Memorandum to Marcus Rhoades. First Section. January 1949

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Introduction

The c^{m-1} locus (hereafter written c-ml for speed in typing) appears to have arisen as the consequence of a change in location of Ds: removal from its former position and insertion into (or close to) a normal C locus. In this new position, it behaves just as it did in its former position. The c to C mutations appear to be associated with its behavior in this new position, as I shall try to point out. In this new location, more has been learned about Ds behavior than could have been learned from Ds in its "standard" location. The position in chromosome 9 where Ds was first discovered will be called the "standard" location. A position of reference is needed because Ds does change its location. In its standard position, it is known that Ds changes its mutation pattern from one general type of behavior to another, i.e., the "early" loss type and the "few-late" loss type of behavior (for details of these two general types, see below). Previously, the behavior of the Ds locus in the "few-late" state had been difficult to analyze and interpret. In its new position at the C locus, various types of behavior of Ds are subject to analysis, as will be indicated.

Secondly, chromosome breakage of a type not previously recognized may be responsible for the mutation phenomena associated with mutable loci. The type of breakage and subsequent fusion of broken ends that will occur in any one "state" of a mutable locus may be controlled by the particular organization of the inserted locus responsible for this breakage phenomenon, at the time of its insertion into a new location. This breakage process need not lead to the simple criss-cross type of fusions as previously suspected, nor to the formation of U-shaped dicentric chromatids already known to arise from mutations occurring in one "state" of the Ds locus. It is probably something quite different. This breakage mechanism will be outlined later. This mechanism should sometimes alter the organization of the inserted locus so that new types of organizations would be formed. These altered organizations will give rise to new "states" of the mutable locus, that is, different rates of production of visible mutations, of stabilization of loci, and of the types of fusions that will occur following breakage. The state of a mutable locus is something more than a quantitative change at a locus. Starting with any one organization that gives a particular series of breakage-fusions events, the types of change to new organizations giving an altered series of events may follow rather definite sequences.

Thirdly, stabilization of a mutating locus may follow as a consequence of loss, during a mitotic cycle, of the inserted locus responsible for the mutation phenomena or of its active component. With some organizations of the inserted locus, each mutation of the mutating locus, with some exceptions, will result in stabilization of the mutating locus. With other organizations, some of the mutations will not result in elimination of the active locus. In these cases, continued mutations will occur. This mutating locus may be a dominant, an intermediate allele or a recessive but mutations will continue.

Fourthly, I suspect that many loci, such as the C locus and the A_1 and A_2 loci are compound loci involving genic action associated with successive or related steps in the conversion of substrate to product. The type of mutations occurring following insertion of a "Ds" locus into such loci will depend upon (1) the position of insertion, (2) the organization of the inserted locus at the time of its insertion and (3) the changes in organization of the Ds locus that will occur subsequently.

If the accumulating data has been interpreted correctly and continues to give further supporting evidence, it will be necessary to change the symbol "Ds" to something more expressive of its basic action. The observed dissociation phenomenon (dicentric chromatid formation) is only one of the consequences of mutations of Ds that occur. The various types of Ds mutation behavior are probably expressions of one basic type of event. This is the important event underlying all expressions of mutability of mutable loci. The newer evidence has made it possible to consider this event more specifically even though there is much that is purely speculative. Nevertheless, the expanded knowledge allows for better directed experimentation and observation.

Fifthly, there are probably a number of unstable dominant loci in my material. These will eventually throw unstable recessives. The wx-ml locus arose from an unstable dominant locus, designated Wx-m. This unstable dominant locus has now been isolated. It will throw more unstable recessives. One such recessive, a new wx-m, is growing in the greenhouse but many more should be detected following suitable crosses now that the unstable dominant that throws unstable recessives has been isolated. The instability of dominants and the unstable recessives they throw are probably all expressions of the one kind of event, i.e., chromosome breakage. Mutable expressions go in a cycle from unstable dominant to unstable recessive to unstable dominant, etc., or to various quantitative levels between full dominant and full recessive. (Full dominant is a relative concept. Super dominants can be isolated.) Stabilization may occur at any quantitatively expressed level, however.

Part I. The c-ml and standard Ds loci compared.

The c-ml locus; general considerations

For the details, the c-ml case may be considered first. A brief review of the behavior of this locus will give the essential facts necessary to an understanding of the discussion. The c-ml locus arose in the cross of a [illegible] yg c sh Bz wx ds ac female plant by a male [illegible] plant that was Yg C Sh Bz wx Ds / Yg C Sh Bz wx Ds, Ac ac. Ten crosses of this male parent were made to c sh ds ac female plants. The ratios were: 2077 C, non-variegated (ac ac ac) : 1199 C to c variegated, early losses (Ac ac ac) : 317 C to c then classified as having the few-late type of Ds action (mainly Ac ac ac) : 1 c to C variegated kernel. This last kernel was obviously not the regular C to c variegation expected as the consequence of Ds action. It showed the opposite type of variegation, from c to C. A plant was grown from this aberrant kernel in the summer of 1947 (plant 4204). The plant was variegated. It showed streaks of yg. This variegation was not unexpected because it was known that both chromosomes 9 in the male parent carried Ds. If Ac were present, just such yg streaks should appear. It was only necessary to determine that Ac was also present in this plant; the later tests proved that plant 4204 was Ac ac. The constitution of this plant was then believed to be yg c sh wx ds / Yg c-ml Sh wx Ds, Ac ac. The data and analysis given below shows that the location of Ds is not as given. Instead, it is probably at, within or close to the C locus. In this plant (4204) no evidence has been obtained for a Ds in the "standard" location.

Summer 1947

Plant 4204 was crossed to:

(1) yg c sh wx ds ac

(2) C sh bz wx ds ac

(3) C Sh wx Ds / C Sh wx Ds, ac ac

(4) pyd c Sh Wx ds / yg c Sh wx ds, ac ac.

From cross (1), it was clear that the male parent possessed two chromosomes 9, one with Yg c-ml Sh wx and one with yg c sh wx. (For kernel counts, see Table 1, Appendix.)

From cross (2) it was clear that a Ds locus was likewise present in the c-ml Sh wx chromosome. ~~About half~~ Many of the Sh kernels (the Ac containing kernels) were C Bz-C bz variegated. (See Table 2, Appendix.) There were few such kernels in the sh class. (This latter observation should have made me suspicious about the location of Ds but I failed to register a reaction. At the time, I did not know that Ds could change its location. Realization of this did not enter my consciousness until late this spring, 1948 following the harvest of the greenhouse crop.

From cross (3) it was likewise clear that a Ds locus was present in the c-ml Sh wx chromosome. Some of the kernels were C to c variegated following Ds mutations in the C Ds chromosome contributed by the female parent; in some of these kernels, the c areas showed c to C ~~mutations~~ variegation due to c to C mutations occurring at the c-ml locus in the chromosomes 9 contributed by the male parent (4204). These latter kernels most always showed homozygous deficient tissues of the type occurring when three Ds loci are present, one in each chromosome 9. The combined evidence indicated quite definitely that a Ds locus was present in the c-ml carrying chromosome of plant 4204.

From cross (1) it was obvious that the expressed mutation rates of c-ml are not alike in all the kernels. The majority of the kernels show relatively few c to C mutations. A small number of Sh kernels, however, had very many c to C mutations. I assumed from this that the kernels showing only a few c to C mutations were either (1) those having a c-ml Sh wx Ds constitution and that the Ds mutations were eliminating the segment of the short arm carrying the c-ml locus from many sectors of these kernels so that the c to C mutations could not occur in these sectors; or (2) that the state of the c-ml locus was low, i.e., few c to C mutations were occurring even in those kernels with c-ml and no Ds (those derived from cross-over chromatids). Again, it was assumed that those kernels with many c to C mutations probably possessed no Ds because Ds could have been removed from the chromosome as a consequence of crossing over between Sh and the assumed position of the Ds locus in the parent plant (4204). Because both chromosomes 9 in the parent carried wx, there was no immediate genetic determination of such a cross-over. The sh kernels showing c to C variegation obviously carried a cross-over chromatid. Barring double crossovers, which are rare, the c-ml chromosome in these kernels should have no Ds locus. These kernels, however, did not show high rates of c to C mutation. That a changed location of Ds had occurred when c-ml arose could not be clearly revealed from an analysis of the crosses made during the summer of 1947. That something was wrong soon became obvious. The nature of the discrepencies will be indicated.

The selection of kernels for tests in the greenhouse last winter centered on establishing the control of c-ml mutation phenomena by Ac. From this crop, it was determined that Ac controlled the c-ml mutations; without Ac, no mutations occurred. It also showed that the time in development when mutations would occur depended upon the dosage of Ac. In this respect, it was quite similar to Ds in response. In the progeny derived from crosses of those plants that were grown from the highly c to C variegated kernels, this greenhouse crop also showed the exact correspondence in patterns of mutation to those already known for one type of Ds. You will remember seeing this similarity in comparing kernels of the constitution C ds / C ds / I Ds, Ac ac ac (and Ac Ac ac) with those kernels having c ds / c ds / c-ml, Ac ac ac (and Ac Ac ac). The two sets of kernels were indistinguishable, respectively, in color pattern of variegation.

The summer planting (1948) included a selected number of Wx kernels showing c to C variegation from cross (4) above. The Yg plants arising from these kernels were all variegated for pyd or for yg. This was not anticipated for I had expected a few of these plants to have a c-ml chromosome with no Ds because of crossing-over between c-ml and wx in the male parent. It did not bother me too much as the sample was small. In order to be sure to eliminate Ds from a c-ml carrying chromosome, some of these plants were crosses (1) to c sh wx ds ac, (2) to C sh bz wx ds ac, and (3) to plants having the rearranged chromosome 9 with c sh Wx and a female-transmissible deficient chromosome 9 carrying Sh and wx but deficient for the C locus. There were other reasons for these crosses but mainly it was to observe the effects of c-ml mutations when Ds was certainly removed from the chromosome. No Ds (standard location) should be present in most of the c-ml Wx chromatids. Only double crossovers would give the constitution c-ml Wx Ds and these are rare.

The results from cross (1) were strikingly different from expectation. Nearly all the Wx kernels that showed c to C variegation were also heavily Wx to wx variegated; this Wx to wx variegation resembled the usual Ds type variegation. In these kernels, the rate of c to C variegation was low, except for sectors in some of the kernels. A few of the Wx kernels, however, were heavily variegated for c to C. These kernels showed only a few wx areas and they usually were quite small. A tabulation of the classes of kernels resulting from cross (1) may interest you for they tell the full story much better than words (table 1). Because the male parent was heterozygous for Ac, only half of the kernels having a c-ml locus could show c to C variegation. Also, the kernels that received the stable c locus (from the parental c Sh Wx chromosome) will not show c to C variegation with or without Ac. You will note that only 14 of the 990 c Wx kernels (no visible c to C mutations) showed Wx -wx variegation whereas all of the 140 c to C variegated kernels that were Wx likewise showed Wx to wx variegation. The presence of detectible sh sectors in some of these heavily Wx-wx variegated kernels made it clear that loss of a segment of the short arm of chromosome 9 was responsible, or often associated with, the appearance of the wx sectors. Some kind of Ds-like action was occurring in these kernels with a c-ml Wx chromosome. This Ds action was not associated with a standard Ds locus in the c Sh Wx chromosome of the male parent. If a Ds locus had been present in this chromosome, many of the c Wx kernels should have been Wx - wx variegated but they were not. Secondly, all the Wx kernels showing c to C variegation likewise

Table 1

$$c\ sh\ wx\ ds\ ac\ ♀\ x\ \frac{c^{m-1}\ Sh\ wx}{c\ sh\ Wx}\ ♂$$

Cross	c → C			c → C	c	Non-var.	
	wx	c wx	c Wx	Wx → wx	Wx→wx	C Wx	C wx
4350-5 x 4434D-3	46	90	141	27	3	1	1
4346-18 x "	40	80	146	18	1	0 ?	0
4349-2 x	34	83	148	22	0	0	1
Totals	120	253	435	67	4 1	1	2

Table 1 (~~extended~~ continued)

Cross	c → C wx	c wx	c Wx	c → C Wx → wx	c Wx→wx	C Wx	C wx
4346-16 x 4434D-5	27	57	71	8	2 ?	0	2
4349-31 x " "	29	82	134	16	3	1	3
4350-18 x " "	10	29	54	6	1	0	1
" -26 x " "	37	99	148	28	0	2*	2
" -34 x " "	34	100	134	15	4 +	0	3
Totals	137	367	541	73	10[1] ✓	3	11

* 1 var. for C-c but peculiar. Late losses, Wx-wx.

+ 2 have defective embryo.

[1] These are probably c → C, potentially. Tests of some non-var. c^{m-1}, Ac carrying kernels have shown var. in the next generation.

showed Wx to wx variegation. These represent the kernels receiving a crossover chromatid that brought c-ml and Wx onto the same chromosome. Since all of these chromosomes show this Ds-like action, a Ds-like locus must have been present in the c-ml Sh wx chromosome of the male parent. Its location must be in, close to or to the left of the c-ml locus. Thirdly, of the 16 C kernels arising from germinal mutation of c-ml, only one showed any C to c variegation and this was a peculiar type. (It will be pointed out later that just such new variegations are to be expected. They represent changed location or changed organizations of the inserted locus responsible for mutable phenomena.) Unless all such C kernels have an ac ac ac constitution, a Ds type variegation should be present in some of them, on the assumption that a Ds is in some position other than the c-ml locus. Fourthly, examination of the C areas in both the c to C, Wx - wx kernels and the c to C wx kernels were likewise instructive. In these kernels, with some expected exceptions, the C areas were not variegated for C to c. In the c to C, Wx - wx kernels, the C areas were Wx, not Wx -wx variegated as would be expected if Ds were present to the left of the C locus. Obviously, then, when a mutation to C occurred, the Ds-like action in this chromosome ceased (with some exceptions, to be considered later).

The combined observations made me suspect that the Ds action was associated with the presence of Ds in the c-ml locus and that the Ds locus was usually lost from the chromosome when a mutation to C occurred. The following conclusions were formulated: that (1) the c-ml locus arose from a C Sh wx Ds chromosome following removal of the Ds locus from its standard location and insertion into the normal C locus; (2) when a mutation of c-ml to C occurs, Ds disappears; (3) the insertion of Ds into the C locus brought about an inhibition in the expression of the genes at the C locus; consequently the modified C locus resembles c in phenotypic expression. (4) When this Ds locus is removed from the chromosome, the normal genic action of the C locus can reappear. (5) Two contrasting types of Ds action can be recognized when Ds is in this new position: (a) one giving the typical dicentric chromatid through breakage and fusion at this new location and (b) one giving the c to C mutations that usually result in no *visible* alteration of the chromosome.

For an understanding of the events underlying mutability phenomena, conclusions (4) and (5) above are most important. They bring up the question: Does Ds in its standard location show these same two contrasting types of behavior? A positive answer to this question is indicated. Two dissimilar types of behavior of Ds in its standard location have long been recognized. The extreme of one type gives many dicentric chromatids as the visible consequence of Ds mutations. The other extreme gives the "few-late" pattern of chromosome segment losses, i. e., few dicentric-forming mutations occur. The relationship between these two contrasting types of mutation behavior of Ds in its standard location will be indicated after some of the supporting evidence for the conclusions (1) to (4) are given. Cross (2) -- C sh bz wx ds ac female by c-ml Sh Bz wx / c Sh Bz Wx, Ac ac male -- will be mentioned first.

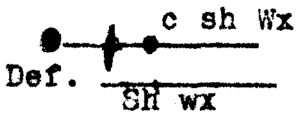
On the ears resulting from cross (2), a number of kernels were variegated for Bz -bz. In those that had the Wx locus, the C bz areas most always were variegated for Wx -wx, i.e., Bz was lost before Wx. This would be expected if dicentric chromatid formation resulted from

Ds mutations at the C locus. (If Ds were in its standard position, the bz areas would be wx.) The results from one such cross are as follows:

C sh bz wx ds ac (Plant 4684-7) x c-ml Sh Bz wx / c Sh Bz Wx, Ac ac (Plant 4434D-5)

C Bz Wx non-var.	C Bz - C bz Wx _wx	C Bz* wx not obviously var.	C Bz -C bz wx
124	12	89	41

The kernels resulting from crosses of type (3) were likewise useful because the classes are those expected on the basis of the conclusions given above. One such cross gave:



ac ac x c-ml Sh wx / c Sh Wx, Ac ac

(Plant 4642-6) (Plant 4434D-5)

3 C Sh Wx non-variegated.

1 C Sh wx non-variegated

11 c to C lightly variegated; Wx-wx variegated; homozygous deficient tissues (from dicentric chromatids following Ds mutations).

19 c to C lightly variegated; wx. Homozygous deficient tissues present (from dicentric chromatid formation resulting from Ds mutations).

5 c to C, heavily variegated; wx. No obvious homozygous deficient tissues. (Such tissues would not be detected if dicentric chromatid formation occurred late.)

60 c Sh wx; no homozygous deficient tissue.

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* Unless early losses of Bz occur, it is often not possible to detect C Bz-C bz variegation. Bz substance from the surrounding Bz areas diffuses into the bz areas giving them the Bz phenotype. A few of the kernels in this ~~class~~ column may belong in the C Bz-C bz class.

43 c to C ~~variegated~~ mostly lightly variegated; Wx; no homozygous deficient tissue

240 c Sh Wx; non-variegated; no homozygous deficient tissue.

The plant arising from the original kernel showing c to C variegation (plant 4204) is now ~~known~~ believed to have had the constitution yg c sh Bz wx ds / Yg c-ml Ds Sh Bz wx ds, Ac ac. Before the transposition of Ds to the C locus was realized, the selection of sh kernels that showed c → C variegation in the cross of this plant to c sh wx ds ac plants, was expected to give kernels having a c-ml locus but no Ds locus. Such c^{m-1} carrying chromosomes represent cross-over chromatids and if Ds had been present at its standard location in the c-ml Sh wx chromosome of plant 4204, they should have no Ds locus. Plants were grown from some of these c → C sh kernels. Crosses of these plants were made to (1) C sh bz wx ds ac plants and to (2) plants having a deficient chromosome 9 (the deficiency included the C locus) Sh wx and a rearranged chromosome 9 with c sh Wx (as well as to c sh ds ac plants). Following cross (1), C Bz - C bz variegated kernels appeared on the ear. Following cross (2), homozygous deficient tissue appeared in the c → C Sh wx kernels. Obviously, the Ds action had not been removed from the c-ml chromosome by crossing-over between c-ml and Sh.

Because the transposed position of Ds in the c-ml chromosome was not suspected this past summer, the most favorable types of crosses to illustrate a new position of Ds were not systematically made. Nevertheless, some additional crosses, made for other purposes, have been helpful because they are consistent with the above interpretation. An illustration of one of such case is given in the cross of a c-ml sh wx / c-ml sh wx, Ac ac male plant to an ac ac female plant that had

IWx

$\frac{\qquad}{\mathrm{IWx}}$ / a terminal deficient chromosome 9 with C Sh wx. This cross gave:

90 I Wx kernels

3 C Wx normal

34 C wx normal; no homozygous deficient tissue

20 C wx, with large amounts of homozygous deficient tissue.

The homozygous deficient tissue in the 20 C wx kernels arises as the consequence of dicentric chromatid formation associated with Ds mutations at the c-ml locus.

There are several points that require further consideration. (1) Why does a stable C locus arise when a c to C mutation occurs? What happens to the Ds locus during such an event? (2) What is the relation between the two visibly different types of events that can occur at the c-ml locus; (a) dicentric chromatid formation and (b) the c to C mutations that do not alter the chromosome morphology? (3) Does Ds in its standard location show these same two main types of behavior? (4) What interrelations exist between these two contrasting behaviors of Ds? (5) What is the evidence for the conclusions that no Ds locus is present at the standard location in the original c-ml Sh wx chromosome? Stabilization of the C locus following a c to C mutation of c-ml will be considered first.

Stabilization of the mutated c-ml locus.

On examining the variegated kernels arising from the first crosses (c sh wx ds ac x 4204), it was noted that the majority of the C areas arising from mutations of c-ml to C were not variegated for C to c, as could be expected. This expectation was based on the assumption that a Ds locus was present in its standard location in the c-ml carrying chromosome of the male parent. If this had been true, 70 to 80 percent of the c to C variegated kernels should have had this Ds locus. Consequently, this absence of C to c variegation in the C areas was unexpected. I wondered at the time whether,in this case, a c to C mutation was associated with a change in Ac activity so that no further Ac controlled mutations would occur in the sector. The evidence for such a conclusion was not substantiated in other crosses. It was known that c to C mutations in one chromosome could occur following Ds mutations in the homologous chromosomes. This was revealed in kernels resulting from the cross of C wx Ds / C wx Ds ac ac female x 4204. It was obvious here that dicentric chromatid-forming Ds mutations could occur in the female chromosomes 9 early in development and that these could be followed in later cell generations by c to C mutations at the c-ml locus in the chromosome 9 contributed by the male.

In the crosses of c sh wx ds ac x 4204, a number of germinal mutations of c to C appeared on the ears. None of these C kernels showed any C to c variegation of the Ds type. If a standard Ds locus were present in the C chromosome of these kernels, many of these C kernels should have shown the Ds activity by giving C to c variegation, for half of them should have an Ac ac ac constitution. 29 C, non-variegated kernels arising from these crosses, and from some similar ones made in the greenhouse last winter, were removed from the ears and plants grown from them in the field this past summer to determine (1) if an Ac locus were present, (2) if Ds were present and (3) if the C locus arising from a c-ml mutation was stable in later generations. Crosses to c ds Ac Ac were made to test (2) and (3);

crosses to c sh wx ds ac were made to test for (2) and (1); and crosses of these plants by I wx Ds ac ac were made to test (1). In all cases, with the exception of the C kernels coming from one particular greenhouse plant (to be considered later) the C loci were completely stable in the presence of Ac. No Ds activity was observed.

It can be concluded, then, that a mutation of c-ml to C frequently produces a stable C locus and that dicentric chromatid-forming Ds activity, previously shown to occur when a c-ml locus is present, usually disappears as a consequence of the mutation phenomena resulting in a C phenotype from a c-ml locus.

Evidence for the conclusion that no Ds locus was present at the standard location in the original c-ml carrying chromosome

Conclusive proof that a Ds in the standard position would be capable of undergoing Ds mutations in C sectors arising from c-ml to C mutations and, therefore, that there was no Ds activity in the standard position in the original c-ml carrying chromosome, came from the following crosses. Plants were obtained with the constitution of chromosomes 9 as follows:

$$\frac{\text{c-ml wx ac}}{\text{c} \quad \text{Wx } \overset{\frown}{\text{Ds Ac}}}.$$

Ac was linked with Ds in the c Wx Ds chromosome (designated by $\overset{\frown}{\text{Ds Ac}}$). These plants were crossed to female c sh wx ds ac plants. A number of the ears have been examined but only 1 has been shelled and counted (table 2). The results from these crosses were obviously so alike that there has been no immediate pressure to obtain large numbers for a table. Because of the linkage of Ds and Ac, most of the c-ml Wx

Table 2

4346-7 x 4664

$c\ sh\ wx\ ds\ ac$ ♀ x $\dfrac{c^{m-1}\ wx\ ds\ ac}{c\ Wx\ Ds\ Ac}$ ♂

c Wx non-var.	c Wx wx	c → C* Wx→wx	c wx non-var.	c → C wx	C → c+ Wx→wx	C wx non-var.
22	128	23	134	13	3	5

* Many C areas are C → c variegated.

+ Germinal C mutations. These kernels are C → c, Wx → wx variegated.

chromosomes should have the constitution c-ml Wx Ds Ac. As table 2 shows, all the c to C kernels with Wx likewise show Wx - wx variegation. In these kernels, the C areas are often variegated for C to c (Ds type). These c areas are wx. Also, in these crosses, all the C Wx kernels (germinal mutations) are C to c variegated with the c areas being wx:—typical Ds type.

There can be no question, then, that Ds mutations can occur in a c-ml carrying chromosome following a c to C mutation if Ds is in its standard location. With Ds at the C locus (c-ml) a mutation of c to C usually eliminates the Ds locus or its action and the newly arising C producing locus is stabilized against further mutation phenomena. Mutations of c-ml to C do not usually result in morphologically altered chromosomes 9. One obtains a normal chromosome 9 with a normal C locus action.* Nevertheless the Ds locus at c-ml shows two main contrasting types of behavior (1) the production of dicentric chromatids and (2) the mutations giving rise to a C phenotype without alteration of the chromosome and usually with elimination of further Ds activity.

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* The use of "normal C locus" must be qualified. Mutations at the c-ml locus have given many apparently normal reacting C loci. There are a number of sub-levels and some super-levels as well as a few resembling the pink mutations (very rare) of c-m2 arising as the consequence of mutations of c-ml. These represent both quantitatively different reaction capacities as well as qualitatively different reaction capacities. According to my present interpretation, such quantitative levels of expression of C activity are to be expected as well as very much less frequent changes that will incorporate adjacent loci (the pink reaction). It would be more profitable to postpone this discussion until after ~~the~~ a mechanism possibly responsible for mutable phenomena has been discussed.

The standard Ds locus: Two contrasting types of behavior

As the last sentence above indicates, it is concluded that Ds activity is responsible not only for the dicentric chromatid formation at the c-m1 locus but also for the c to C mutations of this locus. This conclusion is based upon the striking resemblance between two contrasting types of Ds activity when Ds is in its standard location and the two contrasting types of events occurring at the c-m1 locus. The nature of the evidence for this general conclusion will be considered.

That the Ds locus in its standard position gives two main types of recognizable behavior, has been known for a long time. In an Ac ac ac constitution, Ds loci have been isolated that give many dicentric chromatids as the consequence of mutations of Ds. Kernels with appropriate genic constitutions reveal this clearly. Many of these mutations occur early in development. Such Ds loci have been designated "Ds-early". A contrasting type of Ds behavior results in only a few losses of segments of the short arm of chromosome 9 and they usually occur late. Such Ds loci have been designated "Ds-few-late". In the kernels with a few-late Ds locus, many aberrant types of chromosome breakage occur; the breaks mostly originate at positions other than the Ds locus. The significance of this will be discussed later.

To show that the few-late type of Ds behavior is associated with a particular state (organization) of the Ds locus and that it is not due to modifiers located elsewhere in the chromosome complement, the following crosses were made. Plants were obtained that had the constitution: I Sh Bz Wx Ds-early / I Sh Bz wx Ds-few late, Ac ac. These plants were crossed to female plants with C sh bz wx ds ac.

The types of variegated kernels, with respect to Wx and wx constitutions, were tabulated. The results of these crosses are given in table 3.

Crossing over between Wx and Ds is low, only 1 to 2 or 3%. The 10 I - C bz, wx kernels showing early losses probably arose from crossing over between wx and Ds. The 34 I - C bz, Wx - wx kernels, with the few-late loss pattern of Ds, represent cross-overs between Wx and Ds together with newly arising few-late Ds loci derived from early Ds loci. The few-late type of Ds locus arises rather frequently as a mutation of the early Ds locus but only rarely (none observed as yet) does a Ds-early arise from a Ds-few-late locus. The evidence from table 3 strongly supports the conclusion that the early and the few-late types of variegation reflect two different "states" of the Ds locus. Other observations make this conclusion even stronger. One pertinent observation concerns the appearance of sectors showing the few-late type of Ds behavior in kernels that otherwise show the early type of Ds behavior. If the few-late type of Ds behavior arises rather frequently from the early type of Ds behavior, then such sectors should be seen.

The most instructive evidence for the presence of two such contrasting types of Ds behavior can be obtained from comparisons of these two types of behavior of Ds at the standard location with the same two types of behavior of Ds when at the c-m1 locus. I shall attempt now to point out the similarities.

Table 3

C sh bz wx ds ac ♀ x $\frac{\text{I Sh Bz Wx Ds-early}}{\text{I Sh Bz wx Ds-few-late}}$ Ac ac ♂

Cross	I Wx non-var.	I-C bz Wx wx early losses	I-C bz Wx wx "few-late"	I wx non-var.	I-C bz wx early losses	I-C bz wx "few-late"	Odds*
4363-8 x 4679-2	42	46	4	39	0	33	4
4684-10 x "	60	58	1	73	0	51	1
4362-23 x 4679-3	126	93	15	117	6	114	3
4363-35 x 4679-5	77	72	1	63	2	87	4
4364-1 x 4679-5	120	92	13	120	2	79	5
Totals	425	361	34	412	10	364	17

* The "odds" are kernels showing transposed positions of Ds, that is, I Sh Ds Bz Wx or
I Sh Bz Ds Wx

The relation between the two types of behavior of c-ml and the two types of behavior of Ds at its standard location.

In the cross of a female c sh wx ds ac plant by a male c-ml Sh wx c Sh Wx, Ac ac plant, the Wx kernels showing c to C variegation were of two distinct types. These were considered earlier but will be further considered here. One type was most frequent. It gave very few visible c to C mutations but many mutations leading to dicentric [illegible] chromosomes. There were a few Wx kernels, however, with very many c to C mutations. In these kernels, few dicentric forming mutations occurred for very few wx spots were present. In general the pattern of these wx spots resemble in distribution and size the patterns given by the few-late Ds in its standard location. In both cases, losses of segments of the short arm of chromosome 9 occurred infrequently and [illegible] late in development. With respect to types and frequencies of loss of segments of the short arm of chromosome 9, both can be classified as "Ds-few-late" states. Again, a similarity is found between those c-ml Wx kernels showing low rates of c to C variegation but high rates of dicentric formation with the pattern produced by Ds-early. Furthermore, in some of these kernels, distinct sectors appear with high rates of c to C mutations. These sectors are Wx and have few if any wx spots in them.

To review: The kernels with high rates of c to C mutations give low rates of segmental losses in the short arm of chromosome 9. The pattern of these losses resemble the patterns of losses obtained from the few-late Ds in its standard location. The patterns of losses in the kernels with high rate of loss of segments of the short arm of the c-ml chromosome, resemble the patterns of losses obtained from the standard Ds-early locus. In the former kernels, the sectors with high rates of c to C mutations resemble in frequency and occurrence, the sectors in the latter kernels that show low rates of segmental loss (Ds-few-late sectors). Again, c-ml loci giving high rates of segmental loss throw c-ml loci giving the low rates of segmental loss. Many of these, in turn, have a high rate of c to C mutation. It is concluded, then, that the state of Ds-early in the standard location and the state of c-ml giving high segmental losses represent the same mutational state of Ds whereas the Ds-few-late in the standard position and the c-ml with high rates of c to C mutations represent the same mutational state of Ds. From this, it is concluded that the Ds-few-late, in its standard location, is undergoing many mutations ~~which~~ that are hidden because no visible phenotypic effects follow from such mutations. In order to see this type of mutation behavior of Ds, the Ds locus must be inserted into or close to a locus that will give a *phenotypically detectible* effect, following such a mutation. This occurs when Ds is in the c-ml locus but does not occur when inserted in its standard location.

(See figure 1)

One more resemblance between c-ml and Ds (standard location) should be mentioned. When kernels with high rates of c to C mutations of c-ml are selected and plants grown from them, the high rate of mutation of c to C is retained in later generations. Again, in this respect, the high rate of c to C mutation pattern of c-ml resembles the few-late type of Ds behavior (standard position) because in both cases, mutations back to the dicentric chromatid forming type of Ds behavior occur rarely. The "few-late" type of Ds behavior represents a relatively stable state of a Ds locus.

Figure 1

Comparison of Ds activity at the standard location and at c-ml

Standard Location Ds behavior		c-ml Ds behavior *
Ds - early	Same event	
1) low rate of hidden mutations	⟷	1) low rates of c → C mutation *
a) high rate of visible mutations (segmental losses)	⟷	2) high rates of segmental losses
Ds-few-late	Same event	
1) high rate of hidden mutations	⟷	1) high rate of c → C mutations
2) low rate of segmental losses	⟷	2) low rate of segmental losses

* See note, page 20. Isolates giving low c → C mutation rates but [illegible] directions will be considered at another time.

Comparisons of rates of mutations of c-ml and standard Ds.

It follows from these observations and conclusions that one is in a position to interpret the mutation rates in the kernels with C ds / C ds / I Ds-early, Ac ac ac (or Ac Ac ac, Ac Ac Ac) with those having c / c / c-ml, Ac ac ac (or Ac Ac ac, Ac Ac Ac). Because the color pattern produced by losses of I that occur as a consequence of dicentric forming Ds-early mutations are the same as the color pattern produced by c to C mutations, direct comparisons of mutation rates and patterns can be made. With these particular constitutions, the kernels showing high rates of c to C mutations and those having a Ds-early (standard location) may be indistinguishable in phenotype. Since c-ml loci giving high rates of c to C mutation show relatively few segmental losses and since it has been concluded that the loci have a few-late type Ds locus, it is further concluded that the rates of Ds mutation in both the early Ds state and the few-late Ds state are really the same. Ac controls the time and frequency of some event that occurs at a Ds locus whether or not the consequence of this event leads to a detectable change in phenotype. The mutations resulting in no detectable change in phenotype may be called "hidden" mutations. The presence of hidden mutations will remain a factor causing difficulty in the study of all mutable loci. Their presence is suspected in all the Ac controlled mutable loci. They are a factor that will interfere with any attempt to evaluate mutation rates of any mutable locus. Means of detecting and classifying the types of hidden mutations is absolutely necessary if an understanding of the factors involved in bringing about visible mutations is to be realized.

The Ac control of mutation during development

The similarity in rates of mutations at the c-ml locus, when the c → C rates are high, to segmental losses rates at the standard Ds locus when these are high, has presented an opportunity to determine something about the nature of Ac action in particular cells during development. Why do mutations occur in some of the cells during development and not in others? If two Ac-controlled mutable loci are present in a cell, will a mutation occur at each locus at the same time in the same cell? How could this be determined?

Crosses of plants having c-ml wx ds in chromosome 9 (high rate of c → C mutations) by plants having c (stable) Wx Ds-early (standard location) in chromosome 9 should make this relationship analyzable. If, in the resulting kernels, the c-ml locus in a c-ml wx ds chromosome mutates to C, a C area will arise, the size and position depending upon when and where the c to C mutation occurred, that is, in what cell this occurred during development. If, in this same cell, a mutation occurred at the Ds (standard) locus in the c Wx Ds chromosome, the Wx locus will be deleted and the cells arising following this mutation will be wx. By examining the starch constitution of the cells underlying the C areas in the kernels resulting from such a cross, it should be possible to determine whether or not particular conditions, controlled by Ac, arise in certain cells and whether these conditions affect all Ac controlled mutable loci alike - that is, produce a mutation in all Ac-controlled mutable loci. If so, the C areas should have underlying wx starch constitutions and an exact correspondence in sectors should appear; or some relationship should be visible between the two types of mutations that will tell whether such a coincidence of mutations occurred, (that is, whether one-strand or two strands are involved in the mutation process in a particular cell).

Fortunately, in some of these crosses, the frequency of coincident mutations of c-ml to C in a c-ml carrying chromosome and of Ds mutations in the c Wx Ds chromosome is remarkably high. A large proportion of the C areas have underlying wx starch. This is easily seen in the Ac ac ac constitutions but it is better observed in Ac Ac ac constitutions when a relatively early mutation occurs. In this latter case, the coincidence mutations are very frequent. There are a number of exceptions but such exceptions should be anticipated. This is because several types of hidden mutations occur and also because one of these changes the state of the locus. These newly arising states will give changed rates of visible mutations. Also, the mutation process itself frequently gives rise to exceptional consequences some of which are visible others of which are hidden, as will be shown later.

The high rates of coincident mutations, mentioned above, might lead one to conclude that the Ac control of mutability is related to some particular Ac-controlled condition that arises in particular cells during development. When this condition arises, it will affect a mutation at all Ac controlled mutable loci in this cell whether or not the mutation occurring at any one particular locus will result in a changed phenotypic expression. The Ac locus itself may mutate at the same time also. All of this may well be true but the difficulties in proving it are great. The crosses described above certainly suggest this conclusion regarding Ac control of mutable loci. Nevertheless, other crosses, originally thought to be similar, do not give such a striking coincident rate of visible mutations of these two Ac controlled mutable loci even though many coincident mutations did occur in the development of the kernels on these ears. I am inclined to believe, from observing patterns and rates of visible mutations, that there can be a limited range of states of Ds loci, from ones giving few hidden mutations and many visible mutations to others giving many hidden mutations and few visible mutations. Combinations of c-ml ds / c Wx Ds that are not reciprocally balanced in this respect would show a number of non-coincident visible mutations. This would not mean, however, that coincident mutations did not occur. Rather, it was not possible to see them.

A possible method is available for examining whether or not a range exists in the rates at which these two classes of mutation will occur. It involves various isolates of the c-ml locus. Here, two of the types of mutations of a single Ds locus can be distinguished in c ds/ c ds / c-ml Wx constitutions -- the c to C mutations and the segmental loss mutations. Also, some of the mutations leading to changes in state may be recognized. Selection of c-ml loci giving intermediate frequencies between high c to C and low segmental losses vs. low c to C mutation and high segmental loss can be made. As yet, I do not have available for examination a selection of such c-ml loci with the proper constitutions. However, a comparison of the behavior of the c-ml locus derived from single plants in the crosses of these plants to both (1) c sh wx ds ac and (2) C sh bz ds ac certainly presents suggestive evidence for the presence of such intermediate states of Ds. The c to C rate of mutation following cross (1) has been compared with the C Bz - C bz variegation rate following cross (2). Medium rates of c to C variegation appearing in the kernels following cross (1) are correlated with medium rates of loss mutations in the kernels arising from cross (2). If this proves to be generally true, it will explain why the crosses combining different c-ml wx ds chromosomes with c Wx Ds chromosomes showed different coincident rates of visible mutations of the two loci. If the observed coincident rates are relatively low, the rates of hidden and visible mutations, in the two loci, may not be properly balanced to show the rates of coincident mutations that actually occurred.

The mode of origin of mutable loci

The analysis of the c-ml case has been of considerable interest because it points towards the mode of origin of mutable loci. It is only necessary, it would appear, to insert a Ds locus into (or adjacent to) a normal dominant locus. Inhibition of the dominant locus must be associated with this insertion, if a changed phenotypic expression is to be recognized following insertion. If the organization of the inserted Ds locus gives the "few-late" pattern of behavior, which usually involves loss of the Ds locus as a consequence of a mutation, the inhibitor action will be removed and reestablishment of the normal organization and genic action may occur. Such mutations will occur, of course, only when Ac is present. If this projected mode of origin of mutable loci is the correct one, many newly arising Ac controlled mutable loci should appear in Ac ac and Ac Ac plants that also have Ds loci somewhere in the chromosomal complement. That such newly arising Ac-controlled mutable loci are continually appearing in the Ac Ac and Ac ac plants is well known already. The rate at which such new mutable loci could arise would depend upon the frequency of transposition of Ds loci from one position in the chromosome complement to another position and also upon the relative number of loci present in the complement that can give a changed phenotypic expression following such insertions. The type of mutable phenomena to be first observed after such insertions of Ds loci, would depend on the organization of the Ds locus at the time of its insertion. There is sufficient evidence now to indicate that the rate of transposition of the Ds locus is rather high. This adds considerable strength to the plausibility of this Ds-insertion concept of the origin of new Ac controlled mutable loci.

With regard to changed locations of Ds, the c-ml case has been particularly instructive. It arose in a C Sh wx Ds / C Sh wx Ds, Ac ac plant. This plant had been examined cytologically and was known to have two morphologically normal chromosomes 9 (except in those cells that had undergone a Ds mutation). Among the 4000 tested male gametes of this plant, one possessed a new mutable locus--c-ml. When this chromosome, carrying c-ml, was tested, no Ds activity could be detected at the standard location--the position it was known to occupy in the two chromosomes 9 of the parent plant. Instead, typical Ds activity could be detected at a new location: close to or at the newly arising c-ml locus. No obvious chromosomal alterations appear to have accompanied this change in location of Ds. The chromosomes 9 in plant 4204, were morphologically normal. Also, the c-ml carrying chromosome gave no evidence of reduced transmission through the pollen.

Gross chromosomal alterations may accompany a change in location of Ds. One such case has been analyzed (from plant 4306; for a summary of this analysis, see "Notes to accompany annual report" included with this memorandum). This analysis points towards the mechanism underlying such changes in location of Ds and also supports the conclusion that Ds mutations are associated with some process that often results in tearing out of the Ds locus from the chromosome. This tearing-out process produces broken ends capable of fusing with other broken ends, not only in the torn chromosome but also in the torn-out Ds locus. The torn-out Ds locus, with broken ends capable of fusion with other broken ends, may be inserted into a new location if a coincidental break occurs elsewhere in the chromosome complement. Fusion of unsaturated broken ends, a well established phenomenon, is all that is required to complete the process of change in location of Ds. A second case, possibly quite similar to this one, is now receiving analysis. Other cases involving gross chromosomal alterations that accompany transpositions of Ds which should also add to the evidence on the nature of Ds mutation and transposition, will certainly be found now that I am sensitized to recognize them.

Because of the changed nature of variegation appearing in individual kernels, numerous cases of changes in location of Ds have recently been recognized. Such changes in location of Ds are readily detected in crosses of C sh bz wx ds ac x I Sh Bz Wx Ds, Ac. These transpositions include insertions of Ds to the left of I, between I and Bz or between Bz and Wx to give Ds I Bz Wx, I Ds Bz Wx and I Bz Ds Wx constitutions, respectively. Some of these kernels have been germinated in the greenhouse this winter to obtain lines with these various altered locations of Ds. Unfortunately, germination of some of them did not occur. This suggests that gross chromosomal abnormalities affecting germination capacities may have accompanied the transposition of Ds in some of these cases.

Before considering a possible mechanism responsible for all of the Ds mutation phenomena, it would be profitable to present, briefly, some observations of relatively infrequent but nevertheless very important types of events associated with Ds behavior. These are most easily detected in crosses of C sh bz wx ds ac by I Sh Bz Wx Ds-early or Ds-few-late, Ac males. A change in location of Ds, as mentioned above, is one of these relatively infrequent events. These infrequent events are additional clues to the fundamental mechanism underlying the Ds mutation phenomena. In examining the variegated kernels of the constitution C sh bz wx ds / C sh bz wx ds / I Sh Bz Wx Ds-early, Ac ac ac, some very clear cases of internal chromosome deletion in the Ds carrying chromosome have been observed. These involve (1) deletions of the Wx locus. The resulting sectors are I Bz wx Typical dicentric Ds behavior may or may not be present in such sectors. (2) The deletion may include both Bz and Wx. The resulting sectors are then I bz wx in phenotype. Such sectors may show typical Ds behavior within them or no Ds behavior may be registered. The relative frequency of these deletions is low but their total frequency is sufficiently high to have been observed numerous times. In kernels of the above constitution but having the Ds-few-late instead of the Ds-early, this aberrant behavior is much more frequent per visible mutation then occurs when Ds-early is present. I believe that many of the observed visible mutations produced by the Ds-few-late state are due to such aberrant consequences of Ds mutations.

Without question, the presence of a Ds locus, whether in the Ds-early or Ds-few-late state, introduces chromosomal abnormalities other than those resulting from the more usual types of Ds action. Any hypothesis of Ds behavior must consider the origin of these numerous cases of well defined types of chromosomal abnormalities that occur when Ds and Ac are present but do not occur with Ds ac, ds Ac, or ds ac. The frequency of appearance of these various classes of duplication, deficiency and transposition is too great to be neglected. They must represent some of the consequences of the underlying mechanism associated with Ds mutation phenomena.

The genetic observations of odd types of chromosomal abnormalities accompanying Ds mutations have received cytological confirmation. In plants that were Ds Ds Ac ac, clusters of sporocytes or individual sporocytes have been observed with duplications or internal deficiencies in the short arm of chromosome 9. A few have shown inversions (some pericentric). In some cases, only one full chromosome 9 was present. The other chromosome 9 was represented by a ring-shaped chromosome, usually relatively small. In Ac ac plants having a ds carrying chromosome 9 with a heterochromatic extension at the end of the short arm, and a morphologically normal chromosome 9 carrying Ds, it could be determined that the aberrant chromosome events involved the Ds carrying chromosome and not the ds carrying chromosome. These observations support the conclusion that the aberrant types of chromosomal events are associated with the Ds mutation phenomena itself--something must occur at the Ds locus itself before the observed types of chromosomal rearrangements will arise.

These cytological observations were made early in the study of Ds behavior. They had caused me much concern, even though they were relatively infrequent. Here, again, their frequency was much too high to be ignored. It is realized now that just such conditions are to be expected. It should be possible to obtain from the Ds Ac plants a number of strains with various chromosomal abnormalities. Already, I have obtained plants with duplications and plants with deficiencies in the short arm of chromosome 9 that arose from Ds Ac plants having morphologically normal chromosomes 9. The methods of detecting such plants pertain mainly to abnormalities within the short arm of chromosome 9. However, I do have a case of the involvement of the short arm of chromosome 9 with the long arm of chromosome 8. I have not been looking for such cases. More should be found.

Possible mechanism responsible for mutable phenomena

Enough information has been collected on Ds Ac behavior (1) at the standard location, (2) when transposed to the right of I (case 4306) and (3) when at the C locus (c-ml) to allow consideration of a possible mechanism that could account for the observed types of behavior. These various types of behavior may be enumerated.

In the presence of Ac, and only in the presence of Ac, the Ds locus is associated with different kinds of abnormal chromosome or gene behavior. These various kinds of abnormal behavior surely must arise as the consequence of one fundamental, primary type of event occurring at the Ds locus. It would be difficult to picture a number of different kinds of primary events but it is relatively easy to picture a number of different consequences of one primary type of event. This event must account for the following behavior:

I. Ds at its standard location gives rise to:

1). Dicentric chromatids: the fusion of sister chromatids occurring at the Ds locus. This is the usual event with the Ds-early (state) organization of the Ds locus.

2) The Ds-early state can mutate to the Ds-few-late state by a change at the locus that arises in one cell (at a mitosis?). This is shown by the sectors of Ds-few-late appearing in Ds-early kernels.

These sectors are sharply delimited.

3). Ds-few-late is more stable in organization than Ds-early. This is evident because it maintains the few-late type of mutation pattern in later generations and rarely (if ever) mutates directly back to an organization giving the Ds-early type of mutation pattern.

4). In kernels having Ds-early, a number of internal deficiencies arise that include segments adjacent to the Ds locus. The deleted segments are of various lengths. The most frequent observed deletions are short, although some longer deletions occur. Following such deletions, Ds activity may be retained in the chromosome or it may be lost from the chromosome during the event that gives rise to the deletion.

5). In Ds-few-late, the visible mutations, that is, losses of segments of the short arm of chromosome 9, often involve some event that is definitely not dicentric chromatid formation with fusion of sister chromatids at the Ds locus. The production of internal deficiencies or the production of dicentric chromatids coming from fusions of sister chromatids at positions other than Ds, can occur.

6). The Ds action may be lost from the chromosome completely without altering the chromosome morphology. (The evidence for this when Ds is in its standard location has not been considered up to now. I have obtained some normal chromosomes 9 without Ds that have been derived from Ds Ds Ac ac plants.) This loss of Ds action is interpreted to arise as the consequence of removal of Ds from the chromosome complement altogether or removal of the Ds locus from its standard position and insertion elsewhere. The removal of Ds action following the c to C mutations of c-ml and the removal of Ds from its standard location and insertion into a normal C locus to give c-ml is substantiating evidence for this interpretation.

7). The Ds locus may be removed intact from its standard location and be inserted elsewhere. A spontaneous breakage elsewhere in the complement is probably responsible for the position of insertion, as the analysis of case 4306 shows (see "Notes to accompany Annual Report). In these cases, the Ds locus must be freed from the chromosome completely because it can enter between the two broken ends at the position of the spontaneous break; fusions of broken ends then follow and the Ds locus is now in its new location. The freed Ds locus must have unsaturated broken ends in order that it saturate other broken ends by fusion. Therefore, breakage of some kind must be involved at the Ds locus to give a Ds locus with unsaturated broken ends. The freed Ds locus need not carry with it a large segment of chromosome. In its new location, it need not interfere with crossing over. This is shown in the c-ml case and the 4306 transposition-translocation case. In both cases, crossing over at the region of insertion is not altered. Gross chromosomal abnormalities, arising from various fusions of the several broken ends when spontaneous chromosome rather than chromatid breakage occurs at a locus other than Ds, may accompany a change in location of Ds. The transposition of Ds, in these cases, need not involve a segment of chromatin that carries Ds with it; for it has been shown that Ds can move as a sub-microscopic, independent unit in these cases.

The analysis of changes in location of Ds indicates that the Ds locus may be removed from the chromosome as a sub-microscopic, independent unit and that, as such, it may be inserted elsewhere in the chromosome complement. It indicates, also, that the freed Ds locus has unsaturated broken ends because fusions of unsaturated broken ends must have taken place in the 4306 transfer of Ds. Such fusions would be expected only if the Ds containing, sub-microscopic fragment of chromatin had unsaturated broken ends. The "early" state of Ds (Ds-early), giving many dicentric chromatids as the consequence of Ds mutations, strongly supports a breakage-fusion mechanism as the causative factor underlying Ds -Ac mutation phenomena. On the interpretation that only one kind of event underlies all Ac controlled mutations, regardless of the visible consequences, some form of chromosomal breakage must be suspected as the primary event responsible for mutation phenomena.

II. Ds, transposed to the normal C locus, has given rise to the c-ml mutable locus.

1. In this position, Ds behaves just as it does at the standard location or when transposed to the right of I (case 4306).

a). Ds-early type mutations at c-ml give dicentric chromatids with fusion of sister chromatids occurring at or close to the c-ml locus as the main type of consequence of mutation.

b). This Ds-early state at c-ml may mutate to the Ds-few-late state. When this occurs, dicentric chromatid formation ceases as the most frequent visual consequence of Ds mutations. Instead, the rate of c to C mutations may rise abruptly. The frequency of these c to C mutations may be the same as the frequency of dicentric chromatid forming mutations of standard Ds when this Ds is in the "early" state.

2. c to C mutations of Ds at c-ml result in morphologically normal chromosomes 9.

a). The C locus is usually stable after such an event.

b). The Ds activity ceases following such a c to C mutation. Ds appears to have been lost from the locus altogether as the consequence of the c to C mutation.

3. In the heavily variegated c to C kernels (the c-ml Ds locus equivalent to the standard Ds-few-late locus) where many mutations may be observed, a number of abnormal events have been observed. These observations (progeny test made in 1 case only) suggest that occasionally:

a). The "c-ml" locus (which has the genes of the normal C locus) may be removed from the chromosome along with Ds. This results in a stabilized c locus, no longer capable of mutating to C.

b). The Ds locus may be removed from the c-ml locus and be inserted elsewhere, often in the short arm of chromosome 9. A C phenotype may result but continued Ds activity occurs at the new location. It gives:

(1) Repeated losses of C in the C sectors arising from such an event if Ds is inserted to the right of C.

(2) Losses of C in the C sectors if inserted to the left of the C locus. In this case, however, there are breakage-fusion-bridge cycles that result in twin sectors of c and deep C in c / c /c-ml Ac ac ac constitutions.

These various events suggest that the c to C mutations of c-ml result from some form of chromosome breakage that usually eventuates in loss of the Ds locus from the chromosome. Sometimes, however, the removed Ds locus may be inserted elsewhere if, at the same time that a Ds mutation occurs, a coincidental break occurs elsewhere. The Ds locus may then be inserted between these two newly broken ends.

Mutations of c-ml to C most often restore the full expression of the C locus at one single step, as far as one can state from observations of the color intensities.produced. There are a number of mutations, however, that give rise to much reduced color intensities or occasionally to deeper intensities than a normal C locus. These quantitative changes are part of the evidence that must be considered in any hypothesis relating to the primary event associated with Ds action at the c-ml locus (or elsewhere). The separate hypothesis of quantitative units at a locus offers no difficulties, as seen so far, to a straight forward interpretation of events underlying Ds mutations. If the different quantitative expressions of the various mutations of a single locus are related to some inhibitory factor that can express itself quantitatively, then a new as yet unstudied variable factor will have to be included in the over-all hypothesis. It relates to the nature of genic organization resulting from the primary event but not necessarily to the primary event itself.

What kind of a mechanism will give rise to these various events with the expected relative frequencies? I have thought of one kind of mechanism that does not seem too inconceivable. It involves the following assumptions and interpretations.

1. The main event responsible for all Ac controlled behavior is related to the reduplication of the Ds locus at the time of gene reduplication.

2. Usually, following chromosome reduplication, each new gene molecule lies adjacent to the mother molecule but is not joined to the mother molecule by any chemical bonding.

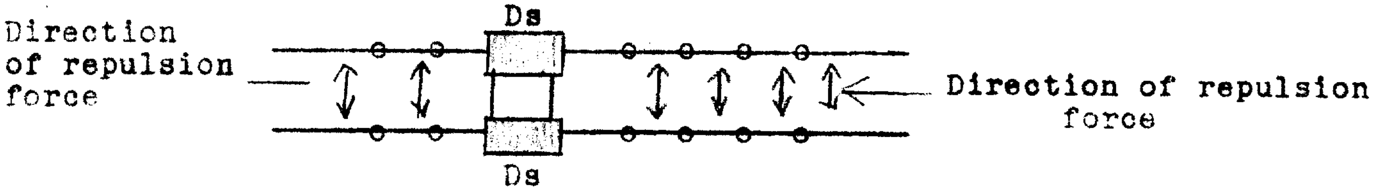
3. Reduplication of gene molecules is orderly in that all the daughter molecules lie adjacent to the mother molecule. Also, all daughter molecules lie in a single plane--that is, the new daughter chromosome is not twisted about the mother chromosome. The behavior of ring-chromosomes during mitosis shows this.

4. During prophase, a repulsion occurs between mother and daughter chromosomes. This repulsion occurs simultaneously or nearly so along the whole chromosome. The mother and daughter chromatids become separated by a rather specific distance, as a consequence of this repulsion force. This is seen by an examination of the two chromatids at somatic prophase. In order to give this precise spacial relationship between two chromatids, some form of repulsion force, following chromosome reduplication, is required.

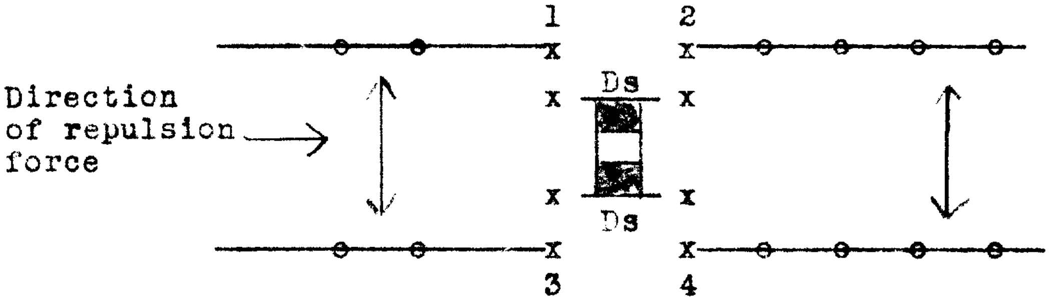
5. When a Ds locus is present in an ac ac constitution, the Ds locus behaves as other loci do during reproduction and thereafter.

6. When both a Ds locus and an Ac locus are present, the mother and daughter Ds molecules remain chemically bonded together following reduplication of the Ds molecules in certain mitoses (controlled by what Ac is doing at the time).

7. When the repulsion force that separates the mother and daughter chromatids sets in, the mother and daughter Ds molecules are still tied together. In the adjacent loci, however, the mother and daughter molecules are free from one another.



8. Because the bonds uniting mother and daughter Ds molecules are still present, a break must occur in this region. The Ds locus may be yanked out of both chromatids:



x = broken ends capable of fusion with other broken ends.

9. Because of the repulsion forces, broken ends 1 and 2 and broken ends 3 and 4 will come to lie near to one another, respectively. Fusion of these broken ends will occur. The Ds locus will be lost to both sister chromatids. A morphologically normal chromosome will result but it will have no Ds locus.

In the case of the few-late type of Ds action, this mechanism seems satisfactory as an explanation. It is necessary to assume that the original insertion of Ds into the C locus, in the case of c-ml, resulted in an inhibition of the action of the C losus. When Ds is removed, by the above mechanism, the original organization of the C locus may again be restored and a normal C action may again occur. This will explain why the majority of c to C mutations at the c-ml locus give rise to morphologically normal chromosomes 9 with stable C loci showing no further Ds activity.

Tension produced by the united Ds molecules may extend along the chromosome during the repulsion of sister chromatids. Breaks may occur not as neatly as diagrammed but may sometimes result in adjacent segments ~~of chromosomes~~ being pulled out of one or both chromatids.

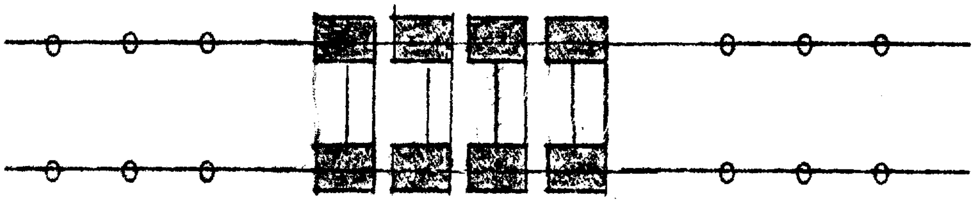
This could give small internal deficiencies in the region of the Ds locus. This may be responsible for the very large number of new recessive mutants that have appeared in the~~se~~ re-stocks.

Again, Ds may be pulled out of one chromatid but not out of the other. Or, the Ds locus may be pulled out of both chromatids but in the process be itself broken. Two Ds loci could then enter one of the sister chromatid~~s~~ if the various broken ends were close to one another. This could give a new, compound Ds locus. Such compounding could continue to build up a rather complex, compound Ds loc~~i~~us. I believe this must occur.

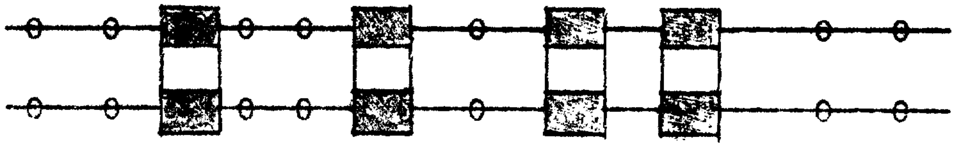
Or, if a coincidental break occurred elsewhere, the free broken ends of the extruded Ds locus might unite with these other broken ends. Transposition of Ds could then occur. If broken ends 3 or 4 were likewise close to the new broken ends, compound, gross chromosomal rearrangements could arise. Various abnormalities could be expected to arise and be visible genetically, when appropriate genic markers are present~~,~~ to make the events realized.

Although this diagrammed scheme seems satisfactory, in general, to account for the few-late Ds behavior it will not tell why one gets the Ds-early behavior, that is, dicentric formation because of fusion of sister chromatids at the Ds locus. As stated above, the few-late type of Ds behavior is rather stable but the early Ds type frequently throws the few-late type. The early type Ds behavior probably involves a more complex organization of the Ds locus, possibly a compound organization with duplicate Ds loci having, therefore, many bonds following gene reduplication.

This may be:



Or, other genes may be present between some of the reduplicated Ds loci:



The extensive binding action of these reduplicated Ds loci may result in rather complex tensions and breakages during the repulsion period that may result in placing broken ends of sister chromatids close to one another so that fusions readily occur. Also, a reversion to a simpler form of Ds organization through deletion of some of the Ds loci could be anticipated, that is, a few-late Ds organization arising from the early-Ds organization.

The various isolates of Ds standard and of c-ml that show grades of Ds behavior between the extreme few-late and the extreme Ds-early, may be fitted into this scheme. The more and the wider separated the Ds loci, the more the chance for dicentric-forming fusions of broken ends and the less the chance for broken ends of the same chromatid to fuse. Also, sequences of change in state of the mutable loci should be anticipated on this mechanism. The high dicentric producers should be the most unstable in maintaining their states whereas the lowest dicentric producers should be more stable in their states, rarely, if ever, throwing a really high dicentric producing state through a single mutation. The states producing intermediate rates of dicentric formation should go in both directions: from intermediate to high or to low. Also, the new unstable mutable loci that arise as the consequence of transpositions of Ds may reflect, to some extent, the state of the locus before the transposition. If a compound locus is transposed, in tact, then the mutation phenomena observed will be quite different from that observed if a single Ds locus enters the new position.

The hypothesis outlined is not complex. It does not call on many assumptions of a purely speculative nature. It is rather one that could well have been anticipated in advance of any suggestive evidence. There is no reason to assume that something could not go wrong with the reduplication process that would lead to just this sort of association of daughter molecule with mother molecule. The aberrant mitotic behavior of ring-shaped chromosomes suggests that something of this nature may go on as an occasional error during the reproductive stage, even in normal loci. It might likewise be responsible for some of the spontaneous breakage phenomena known to occur rather frequently in the maize chromosomes. It is the Ds - Ac combination that brings this to frequent expression.

Part II. The c-m2 locus

Classification and description of the types of mutations occurring at the c-m2 locus

Mutations occurring at the c-m2 locus differ from those occurring at the c-ml locus in several easily distinguishable respects. The c-m2 locus is Ac controlled; mutations occur only when Ac is present. Also, the time and frequency of mutations of c-m2 respond to Ac dosages as do c-ml and the standard Ds.

The mutations of c-m2 may be classified into several catrgories:

I. The Pink mutations. These give various grades of pink to red color in the aleurone with pr pr constitutions or various grades of purple color with Pr. The term "pink" is laboratory language. It is not a well chosen descriptive term. Until I know more about the various classes of c-m2 mutations, I shall not try to coin specific designations. I shall use the term "pink" to cover a series of mutations that appear to be similar or related. Later, it may be necessary to distinguish between various subclasses of this group.

a). There is a wide range in the intensities of the color produced following mutations of c-m2 to pink. Some mutations to pink are too faint for the outlines of the sectors to be certainly defined. Some of these "hidden" mutations may be detected when special conditions are present. These will be described. Other pink mutations are quite dark. A quantitative series of pink alleles are produced by mutations of c-m2. These quantitative alleles of pink may be obtained and maintained from isolates of germinal mutations. Only a limited series of such alleles have been isolated and studied in later generations but many more are available for such purposes. The germinal pink isolates that have been studied so far have been relatively stable in their expression. I suspect, however, that some of these germinal mutations may respond to Ac by giving variegation in the depth of color. I am not too sure of this as yet because the pink phenotype in these isolates does not show uniform distribution of color over the aleurone even in ac ac ac constitutions. It is somewhat mottled although the grades of color contrasts in a single kernel are not extreme. The Ac ac ac constitutions seem to be more mottled and in patterns suggesting changes at the locus. I don't know now whether this effect is due to changes at the pink locus or to changes at other, as yet non-detected Ac-controlled loci.

b). The pink mutants are often associated with the production of some diffusible colorless substance. This substance (substance 1) can be used by a normal C locus to increase the intensity of the aleurone color in those cells having a normal C locus. Apparently, relatively large amounts of this diffusible substance may be utilized by single cells having a single normal C locus. When this utilization occurs, the color of the aleurone in these cells may become very deep, giving a super-C color intensity. The dosage responses of a normal C locus may be reflections of the limited quantities of this substance that one normal C locus produces. The methods of showing the presence of this diffusible colorless substance produced by cells having mutations to pink, involves an analysis of the color patterns and distributions in sectors of kernels resulting from the following combinations of loci. These combinations will also show that the colorless diffusible substance is not produced by an unmutated c-m2 locus.

(1). c-m2 female x C-normal Ds male; Ac ac ac or Ac Ac ac constitution

(2). " " x chromosome 9 with a broken end. This broken chromosome carries a normal C locus. Ac ac ac or Ac Ac ac constitutions.

(3). " " x c-ml male; Ac ac ac or Ac Ac Ac.

(4). pink " x C-normal Ds male. Ac ac ac or Ac Ac ac.

(5). " " x chromosome 9 with broken end. This broken chromosome carries a normal C locus. ac ac ac constitutions as good as those with Ac.

(6). " " x c-ml male. Ac ac ac or Ac Ac ac.

The color patterns produced following each of these crosses will be described later.

c). The isolated germinal mutations to pink have given dosage responses. This has not been extensively worked out but the grades of intensities of pink color in selfed ears of plants with pink / c compared with intensities given by crosses of the same plant to c female plants, certainly suggests dosage effects. More instructive for the dosage studies of pink are the kernels with c / c / and a chromosome 9 carrying pink that has a broken end. The resulting breakage-fusion-bridge cycles in the pink carrying chromosome produce definite patterns of changed color intensities, obviously associated with different dosages of the pink locus. Too few kernels are available for an extensive analysis but those examined clearly show dosage effects. They indicate that the more pink loci present, the deeper the color. The quality of the color and the intensities in the sectors with various doses of pink differ from those produced by similar unit doses of a normal C locus.

d). The pink mutants (some of them, at least) are deficient for some substance that a normal C locus produces. Two such substances may be deficient but at least one is a colorless, diffusible substance (substance 2) that is associated with the activity of a normal C locus. The cells with the pink mutation can use this substance to increase the intensity of the pink color. This has been shown by an analysis of color patterns and distributions in the sectors of the kernels having constitutions given above. In addition, the combination of c-m2 female x C-normal ds male, Ac ac ac or Ac Ac ac, has been useful.

II. Mutations of c-m2 giving conditions that resemble the full C genic activity although varying in quantitative expression of this activity.

In crosses of c ac females x c-m2 Ac Ac males, pink sectors appear in the kernels of the resulting ear. Within some of these sectors, mutations to a type of full C expression often occur. Unless there has been a duplication of the c-m2 locus at the same time that a mutation to pink occurred to give two c-m2 loci, each capable of independent mutation, it may be concluded that a mutation of a single c-m2 locus to pink can be followed by a second mutation at this locus to give a full C type expression. None of the germinal pink mutations that have been carried to a second generation have continued to give these pink to full C mutations in Ac constitutions. The selection for continued study of germinal pink mutants with spots of full C activity in them, has come only from the crosses of c-m2 females x c males. In these cases, the female contributed two loci to the endosperm (two chromosomes 9).

A pink mutation may have been present in only one of them. The other locus might have been a non-mutated c-m2 locus and capable of mutating to full C. This could give C spots in a pink background. The evidence for this is suggestive. In 4 of the 7 tested cases (4451A, 4456-2, 4456-4, 4458A-2) the chromosome entering the egg nucleus likewise carried pink. In the other 3 cases, the chromosome entering the egg nucleus carried a non- mutated c-m2 locus (4456 -1, 4456-3, 4458A-1). If the mutation to pink occurred during the divisions of the female gametophyte, just such apparently conflicting results could be anticipated. Such a female gametophyte could have nuclei with the pink mutation and nuclei with the unmutated c-m2 locus. Because mutations of all Ac controlled mutable loci usually occur late in the development of the sporophytic tissues or often not until the gametophyte or endosperm stage is reached, the period of origin of germinal mutations could be as late as that just described. (It might be mentioned at this time that occasionally an Ac controlled mutable locus may mutate early in the development of the spotophytic tissues. It is a very rare event, however.) Tests of the stabilization of pink mutations must come from the reciprocal cross: c x c-m2. The germinal pink mutations showing full C spots must be selected and tested. They occur but this obviously critical test has not been made. I was not sharply in focus on the c-m2 mutations when the selection of germinal mutations was made!

A single (?) mutation of the c-m2 locus to give the two diffusible substances or at least a second diffusible substance (substance 2) that is associated with gene activity of a normal C locus can occur. Well defined sectors, assumed to have substance 1 and known to have substance 2, appear on the kernels coming from the following crosses:

(1) c-m2 / c-m2, Ac ac; self-pollinated.

(2) c-m2 female x c male. (Ac ac ac or Ac Ac ac constitutions)

(3) c female x c-m2 male. (" " " ")

(4) pink female x c-m2 male and reciprocal. (Ac ac ac or Ac Ac ac constitutions).

It is concluded that if not a single mutation then a single event at the c-m2 locus may result in a modification of genic organization that will produce both of these substances associated with action of a normal C locus. The amounts of the substances produced need not be at the same levels as those produced by a normal C locus. The levels may be considerably lower if the color intensities shown are any indication of such levels.

As stated above, it is possible that successive mutations occur, first to pink, which often produces an excess of substance 1, and then to a mutant giving a darker color associated with the production of some substance 2. There is no certain evidence as yet that mutations giving substance 1 are produced without some accompanying pink color formation, however faint this pink color may be. There is some evidence suggesting that mutations do occur that give substance 2 but not substance 1. The sectors suggesting this are pink with deeper colored rims in the pink sector on a restricted part of the border between a pink and a colorless sector; or, within a pink sector, darker areas with diffuse borders often appear. I am not at all certain that this interpretation is the only one that will explain some of the color patterns of these sectors. More observations and thought are required.

The mutations to full C activity are quantitatively expressed in that various grades of color intensities are present. The sectors with these phenotypes may be quite light in color or fairly dark. No germinal mutations to full C color activity have been detected so far. This is not evidence that they do not occur. I have not looked exhaustively for them. Unless they give deep grades of full C color, I might confuse them with pink. I know now that, often, the two differ in appearance, regardless of color intensit~~ies~~y expressions. It should be possible to discriminate between the two, in some cases at least. I have not taken time to find them but will do so.

III. The unmutated c-m2 locus produces no detectible quantities of either substance 1 or substance 2. This will be shown when the kernels resulting from the crosses enumerated above are described.

IV. Hidden mutations occur at the c-m2 locus.

The frequency of hidden mutations seems to be quite high. This is shown by:

(1) The types of sectors produced in the cross of c ac females x c-m2 Ac Ac males. When the variegation pattern in these kernels is compared with the variegation pattern produced by c-ml (from the heavy visible-mutation producers) or with those produced by Ds-early, it is evident that the visible sectors in the c-m2 kernels represent some form of sub-sector arising in a descendant cell of one that had undergone some primary event. This original mutation event in the ancestor cell resulted in a visibly changed phenotype only in a sub-sector or sub-sectors. Either some kind of (a) segregation to daughter cells occurs following the initial event or (b) successive mutations follow after the original mutation or (c) both types of events occur.

(2). Mutations of c-m2 to stable c loci occur rather frequently, I suspect. Some stable c loci have been isolated from crosses involving c-m2 / c-m2, Ac Ac or Ac ac plants. I can not say that all such mutations are completely stable. Several have been carried for three generations without showing mutation in the presence of Ac. Several ears have appeared in the crosses of c / c-m2, Ac ac females x c, ac males that show large sectors on the ear with only colorless kernels. This may be due to loss of the Ac locus (evidence for loss of Ac will be presented later; it is not uncommon;) or to stabilization of the c-m2 locus. Evidence that will differentiate between these two alternatives will be easy to obtain. It will be necessary, in any case, to determine whether mutations of c-m2 to stable c are associated with some segregation of chromatids, i.e., stable c in one chromatid and an altered organization of the c-m2 locus in the sister chromatid. The sub-sectors, mentioned above, suggest segregations may sometimes occur.

(3) Some "hidden" mutations are actually pseudo-hidden in that they are pink mutations with such faint colors that detection may be very difficult or not possible, in many cases.

(4) I suspect that c-m2, as well as c-ml, has a class composed of several kinds of hidden mutations that are not easy to detect. These include changes in organization of the c-m2 locus that will result in changes in the frequency and type of future mutation; losses of parts of the c-m2 locus; duplication of parts; relocation of parts, etc. Although c-m2 may illuminate some of the activities of a normal C locus, a study of its mutable behavior is discouragingly complex when one has this locus as well as the other mutable loci to carry along.

V. Mutations at the c-m2 locus have not resulted in many obvious losses of segments of the short arm of chromosome 9. The genetic tests for this are inadequate, however. The crosses that could show this clearly involve c-m2 Wx to:

(1) c sh wx ds ac and to (2) C sh bz wx ds ac. The Wx-m locus was carried by the chromosome with c-m2. The wx areas in the kernels coming from cross (1) are usually due to mutations of Wx-m. In cross (2), only a few kernels with C Bz - C bz areas were observed. These areas may arise from events other than those associated with the c-m2 mutations. It may be stated, at least, that the isolates of the c-m2 locus have not given, as yet, any states that regularly result in loss of segments of the short arm of chromosome 9. I believe they will appear, sooner or later.

To summarize:-

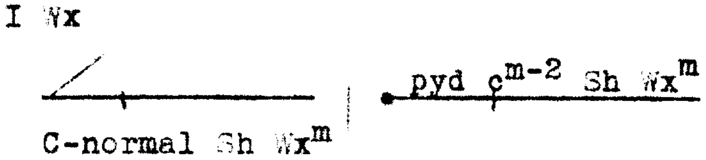
The c-m2 locus may be compound in that the mutations occurring at c-m2 are not associated with the quantitative expression of one type of reaction. Two distinctly different types of visible mutation occur at c-m2. They involve the production of two different diffusible, colorless substances, both necessary for full C expression. The production of substance 1 is often associated with the appearance of some pink color. Mutations giving both substance 1 and substance 2 may occur. Although both substances may be present in a sector arising from a cell having such a mutation, full C color need not appear in this sector. Quantitative expressions of full C activity are observed. The mutations giving pink likewise show quantitative expressions. Some mutations to pink give rise to stable loci that no longer mutate in the presence of Ac. Others may be mutable but this is not certain as yet. Mutations to full C type activity, regardless of the quantitative level, result in production of some diffusible substance (substance 2) that the pink locus can use to increase its color intensity. This same substance is likewise produced by a normal C locus.

That the weakened color intensity of pink mutations appears to be associated with some specific deficiency of a needed substance is suggested by the dosage responses of the pink mutations. This substance can not be substance 1 for substance 1 is often produced in excess even in the mutations giving very faint pink. It may be substance 2. If so, then all mutations giving pink must likewise produce some substance 2. Neither substance 1 nor substance 2 is produced by the unmutated c-m2 locus, however. If the intensity of the pink color is an expression of the level of some limiting substance this substance must be one of the products of mutations of c-m2. It is not controlled by other loci, as the dosage responses of pink indicate. This may be substance 2, as mentioned above, or it may be a third substance associated with genic action of a normal C locus. Although this substance-producer interpretation of mutations of c-m2 fits the observations so far made, I believe that one should be canny about accepting it as it has been presented. I have that uncomfortable feeling of having mentally over-looked the missing link that could simplify the whole interpretation. This is by way of confession and not of retraction.

Before illustrating the color patterns produced in the kernels coming from the enumerated crosses, some statements should be given regarding the origin of c-m2.

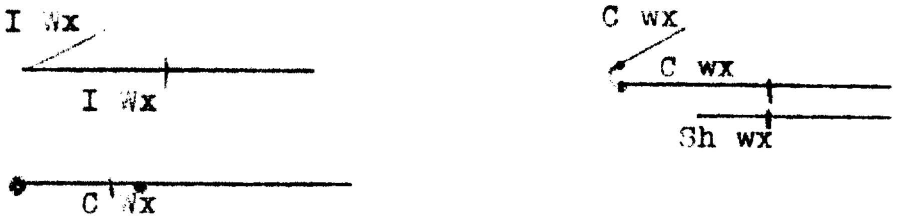
The origin of the c-m2 locus

The c-m2 locus first appeared on an ear of a self-pollinated plant--plant 4000B-2. This plant had the constitutions:



, Ac Ac. It is only in this one plant of the culture that I have been able to find any evidence for a mutable c locus. Also, I have not been able to determine, as yet, whether or not a mutable C locus was responsible for the first appearance of this c-m2 locus, or whether it came from a normal C locus, as did c-ml. All that I can state is that there is no certain evidence for its origin from a C-m locus. Culture 4000 was segregating a mutable pale-green locus. Each plant in the culture was self-pollinated to determine whether or not the pale-green mutable locus was present. The origin of plant 4000B-2, that had the c-m2 locus, will be given starting as far back as the cross that give rise to a kernel ~~with~~ that had received two broken chromosomes 9 in the zygote.

(1) 2467A-5 x 2476-6



One I-C; Wx-x kernel was selected from this cross because both chromosomes 9 had newly arising broken ends terminating the short arm. This kernel gave plant:

Summer 1944
B-series

(2) 42-B. The main ear and the tiller ear of plant B-42 were selfed. The tiller ear segregated the new mutable pale-green locus. The progeny from selfing the main ear did not give this mutable locus.

(3) The progeny of the tiller ear gave culture 3592. This culture segregated pyd as well as the new mutable pale-green locus. Cytological examination of a number of plants in this culture were made and genetic tests confirmed the constitution of the tiller of plant 42-B to have a chromosome 9 with a duplication of the short arm and carrying I Wx and a morphologically normal chromosome 9 with pyd C Sh wx. Ac was also present in culture 3592--whether homozygous or segregating, I do not know.

(4) Plant 3592A-9 was selfed. It had a duplication chromosome 9 with I Wx and a normal chromosome 9 with pyd C Sh wx. The progeny of this self gave:

(5) Culture 4000. 4000B came from the I Wx y kernels.

Selfing 4000B-2 gave the c-m2 locus carried by the morphologically normal chromosome 9 of this plant. None of the other plants in culture 4000 showed the presence of a c-m2 locus.

Examination of the selfed ears of other 3592 plants has given no indication of a c-m2 locus. These plants, however, did show other mutable loci. Those recognized were:

a). y to Y

b). Starch consistency change from wx-like to Wx but the wx-like starch was not associated with wx-staining reaction. Possibly the areas with Wx-like starch consistency stain a deeper blue than normal Wx. I am not certain that all these areas show this. It has received only a cursory examination.

c). Pale-green seedlings mutating to normal green.

d). A Ds-few-late type of behavior in one plant.

e). In plant 4000B-2, a Wx-m locus was present. The tests could not show for certain whether this Wx-m locus was likewise present in other plants of the culture. I think it must be from the constitution of plant 4000B-2. It could be found out, though.

Obviously, the parent culture~~s~~ from which plant 4000B-2 arose was full of mutable loci. Except for c-m2 and Wx-m, both of which are Ac controlled, it is not known whether or not these mutable loci are Ac controlled. b) and d) above both look like Ac controlled mutable loci from the patterns of variegation seen in the examined kernels.

Appendix. Table 1

$$c\ sh\ \text{♀}\ x\ 4204\left(\frac{\underline{Yg}\ c^{m-1}\ Sh\ \overset{Bz}{}\ wx}{yg\ c^{s}\ sh\ \underset{Bz}{}\ wx}\ \ Ac\ ac\right)\text{♂}$$

Cross	Germinal mutations	c^{m-1} var.		No C Spots	
	C Sh	$c \rightarrow C$ Sh	$c \rightarrow C$ sh	c Sh	c sh
4074-21 x 4204	1	66	2	81	158
4075-9 x "	3	55	4	93	183
4074-18 x "	5	85	1	117	233
4145C-1 x "	0	33	1	45	69
4078-6 x "	1	65	1	96	169
4081-28 x "	2	54	1	92	154
4074-5 x "	5	70	1	82	195
4081-25 x "	5	51	1	79	162
4081-30 x "	1	30	3	40	136
Totals	23	509	15	725	1453

Total = 2725
1257 Sh : 1468 sh

Appendix Table 2

C sh bz wx ds ac♀x 4204 $\dfrac{c^{m-1}\ \underline{Ds}\ Sh\ \overset{Bz}{\,}wx\ ds}{c^{s}\ sh\ \underset{Bz}{\,}wx\ ds}$ Ac ac ♂

Cross	C Sh Bz non-var.	C Bz-C bz Sh	C sh Bz non-var.	C Bz-C bz sh
4083-3 x 4204	117	76	213	6
" x "	110	58	184	3
" -12 x "	64	37	90	2
Totals	291*	171	487	11

* Some of these kernels may have c^{m-1} and Bz but not be classifiable because of:

a) heavy c — ^{C} mutations. Such a state of the c^{m-1} locus gives few dissociation mutations

b) late dissociation mutations. These will give too small areas for bz to show due to diffusion of Bz substance from surrounding Bz cells.

Appendix Table 3

$c\ sh\ ds\ ac$ ♀ x $\dfrac{c^{m-1}\ Sh}{c^{s}\ sh}$ Ac ac ♂

F_1 kernels tested for ac constitution and c-m

Selected F_1 kernels → Cross of F_1 Plants from selected kernels	c — C Sh kernels Ac ac	ac ac	c Sh Ac ac	ac ac	c sh Ac ac	ac ac	Origin of ♀ culture
4421 x C Ds ac	B 7	2*	D 2	7	E 10	6	4074-5x4204
4422Bx "	8	0	-	-	--	-	4075-9x "
4423 x "	B 8	0	D 3	18	E+1D 6	10	4074-18x "
4425Cx "	6	0	-	-	-	-	4081-25x "
4426Bx "	7	0					4081-28x "
Totals	36	2	5	25	16	16	

* = 4421B-8 $\underline{c}^{m-1}$ $\underline{ac\ ac}$

Plants from F_1 c Sh kernels x c^s Ac Ac

	c — C	c non-var.
4421D x c^s Ac Ac	7	2
4423D x " "	12	4

Appendix Tables ~~and~~ 4, 5, 6

c sh wx ds ac♀x c^{m-1} Sh / c^{m-1} sh, Ac Ac ♂

Table 4

Cross	C Sh	C sh	c→C Sh	c→C sh	c Sh	c sh
					no C spots	
$4323\text{-}7^{I}$ x 4294C-1	2	1	167	153	11	8

c^{m-1}_{Sh} / c^{s} sh ac ac♀x c^{s} sh Ac Ac ♂

Table 5

4294C-1x4288-2	0	0	38	1	2	37

c sh ac ac♀x $\dfrac{\bullet\!-\!c^{m-1}\ Sh}{-Sh}$ Ac ac ♂

Table 6

Cross	C	c → C	non-var.
$4293\text{-}2^{I}$x4295-1	2	103	99
" x " -3	9	119	138